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# CHANGES IN RIBONUCLEASE ACTIVITY RESULTING FROM STEM INFECTIONS OF WESTERN WHITE PINE BY BLISTER RUST

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ABSTRACT

*Ribonuclease activities in cell-free extracts of western white pine stem tissues infected by blister rust were determined within and near active infection centers. Enzyme activity of samples taken from the infection centers, the actively sporulating margins, newly infected margins, and from bark tissues well in advance of infection showed characteristic changes in catalytic properties. These included changes in specific activity, in substrate specificity, and in heat stability.*

KEYWORDS: ribonuclease, white pine, blister rust, host-parasite interaction, physiology, *Pinus monticola, Cronartium ribicola.*

The efficiency of ribonuclease extraction and assay procedures (Tang and Maretzki 1970; Udvardy and others 1969; Wyen and others 1969; Wilson 1967, 1968) and the demonstrable effect on this enzyme system by parasitism of blister rust (*Cronartium ribicola* J.C. Fisch.) in pine tissue cultures and *Ribes* leaves (Harvey and others 1974) and other rust fungi on their respective hosts (Scrubb and others 1972; Rohringer and others 1961) suggested similar changes might occur in pine stem infections. This research was undertaken to document changes in ribonuclease activity in blister-rust-infected pine seedling stems. These measurements provide a basis for comparing host-parasite interactions in a tissue culture environment (Harvey and Grasham 1969) with those of a natural system.

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## MATERIALS AND METHODS

Seedlings of western white pine (*Pinus monticola* Dougl.) were derived from open pollinated seed collected in the St. Joe National Forest in northern Idaho. These were grown in nursery beds for 8 years. Both healthy and infected plants came from blocks of the same planting. Three years prior to sampling, part were inoculated according to the following procedure. Five-year-old seedlings in nursery beds were enclosed in wood framing that extended 25 cm above their growing tips. This framing was covered with a coarse wire mesh. After fogging of the enclosed seedlings with tapwater, teliospore-bearing leaves from western black currant (*Ribes petiolare* L.), collected in the St. Joe National Forest in September, were placed on the wire mesh spore side down. The leaves were, in turn, covered with several layers of burlap cloth kept moist throughout a 72-hour inoculation period.

Samples for ribonuclease (RNase) extraction were obtained by excising cortex tissues from the following locations at or above the center of single infections from 20 stems and tissues of the same age from 10 healthy stems (collected in May 1972). Infected tissues were obtained from (1) the innermost to the outermost (nonnecrotic) edges of the aeciospore-bearing region (insofar as possible, spores were gently removed prior to extraction with a soft plastic brush), (2) the innermost to the outermost limits of the nonsporulating region (determined by the yellow discoloration), (3) 7-mm strips of tissues whose inner edge was 3 mm and outer edge was 10 mm beyond the yellow margin, and (4) 3-cm strips of tissues whose inner edge was 2 cm and outer edge was 5 cm beyond the yellow margin of the rust infections. All samples were divided into 5-g lots and frozen immediately after collection.

All possible care was exercised to prevent contamination of the RNase between samples or with that from any external source. Handling of tissues in preparation for and of samples during extraction was accomplished with scrupulously cleaned instruments and vessels while wearing disposable plastic or latex gloves.

For extraction, 5 g (fresh weight) of excised pine-stem cortex tissues were suspended in 25 ml of ice-cold, 5-mm potassium phosphate extraction buffer, pH 6.7, containing 2 g polyvinylpyrolidone (PVP Sigma Chemical Co.). This mixture was homogenized with an Omni Mixer (Sorvall, Inc.) set at high speed for six 30-second periods, with 30 seconds between runs. The stainless steel homogenizer vessel was immersed in an ice-water bath throughout. The homogenate was centrifuged (5° C) for 30 minutes (10,000 rpm) in a refrigerated centrifuge (Sorvall RC2-B). The supernatent was dialyzed immediately (5° C) according to the following schedule: 2 hours in 400 ml, 4 hours in 1,000 ml, and 24 hours in 2,000 ml of extraction buffer. The enzyme concentrate was used directly for protein estimations, RNase determinations, and measurement of potential hydrolytic activity by Deoxyribonuclease (DNase), phosphodiesterase, alkaline, and acid phosphatase (Scrubb and others 1972).

All assay procedures were as described previously (Harvey and others 1974; Scrubb and others 1972). The pH optimum for the reaction mixture was 4.5.

One unit of RNase is herein defined as the quantity of enzyme catalyzing an increase in $A_{260nm}$ of 1.0 under the standard conditions of assay. Specific activity of RNase is described in units/mg protein. The specific activities of other hydrolases are expressed as $\Delta$OD at the appropriate wavelength/mg protein.

## RESULTS AND DISCUSSION

Initially, estimating protein in pine stem tissues, particularly infected tissues, proved difficult. This problem was related to the presence of dialyzable extractives, probably low molecular weight phenolics, that caused excessive color in extracts at the $280/260_{nm}$ range. Extensive dialysis, as noted in the methods, resolved the problem.

Although the conditions prevailing during extraction and in the RNase reaction vessels were generally unfavorable to hydrolytic contributions from DNase, phosphodiesterase, alkaline and acid phosphatase, their potential activity was measured. Table 1 summarizes typical values for these enzymes when tissue extracts were assayed under optimum conditions for each. Even under these conditions the rates of hydrolytic activity in infected tissue extracts were lower than in healthy tissue extracts. This is a clear indication that these enzymes did not contribute substantially to ribonucleic acid (RNA) hydrolysis.

In a test to determine the effect of mechanical injury, healthy stem cortex tissues were sectioned into 2 mm squares, then incubated for 12 hours prior to extraction. No changes in RNase or heat stability resulted. The differences in the specific activities and heat sensitivities of RNase extracts from healthy and from locations in and near infections of pine-seedling stems showed a definite pattern of changes (fig. 1). Changes in the substrate specificity of RNase extracts showed a pattern of change resulting in similar specificities in all tissues under the influence of the parasite (table 2).

Table 1.--*Changes in the activities of enzymes (other than ribonuclease), capable of hydrolyzing the phosphodiester bond, from healthy and blister-rust infected pine-stem cortex*

| | Hydrolytic enzyme ΔOD at appropriate wavelength per mg protein | | | |
|---|---|---|---|---|
| Tissue | DNase | Acid phosphatase | Alkaline phosphatase | Phospho-diesterase |
| Healthy pine stem | 10.0 | 15.0 | 16.2 | 0.0 |
| Infected pine stem | 5.5 | 12.0 | 9.5 | 0.0 |

Table 2.--*Hydrolysis of $^{3}H$-labeled polynucleotides by RNase extracts from blister rust-infected and healthy pine-stem cortex tissues. Infected samples taken from (1) between 3 and 30 mm beyond the discolored zones above infected areas, and (2) from within the discolored region excluding the sporulating area*

| | Hydrolysis dpm/mg protein | | | | |
|---|---|---|---|---|---|
| Tissue | Poly A | Poly C | Poly U | Total dpm | Preference |
| Healthy | 19,035 | 35,142 | 0 | 54,177 | C>A>U |
| 3-30 mm | 21,833 | 15,916 | 0 | 37,749 | A>C>U |
| Infected | 39,924 | 3,166 | 0 | 43,090 | A>C>U |

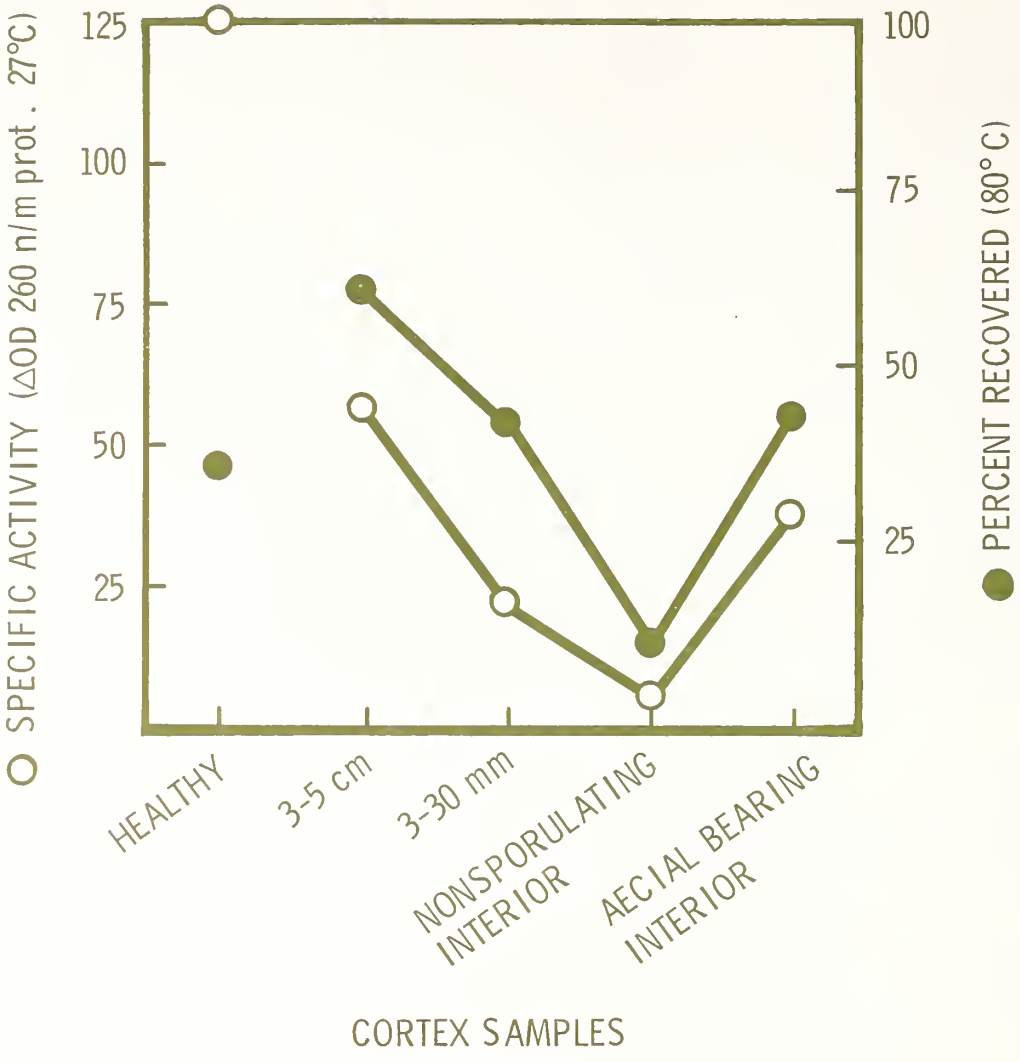


*Figure 1.--Soluble RNase specific activity and respective temperature stabilities from healthy and infected pine-stem cortex. Specific activity expressed in* $\Delta OD\ 260_{nm/mg}$ *protein at 27° C. Temperature sensitivity determined by heating the enzyme solution to 80° C for 10 minutes, then cooling in an ice bath prior to adding the substrate.*

## CONCLUSIONS

The data show a strong similarity to the *in vitro* and *in vivo* systems used previously. They are consistent with the following generalizations regarding white pine-blister rust interactions:

1. All infected tissues (pine stems, *Ribes* leaves, and pine-tissue cultures) contain a unique RNase present in neither healthy nor mechanically injured tissues (this paper; Harvey and others 1974).

2. Many changes are apparent in tissues adjacent to, but not yet penetrated by this parasite (this paper; Harvey and others 1974; Robb and others 1974).

The results support the hypothesis that axenic culture systems provide a valid tool with which to dissect a host-parasite combination in order to study both the components and their interactions.

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